Editorials

Footnote to the First Year

ONE YEAR AGO the Western Research Societies decided at their annual Carmel meetings to inaugurate the Clinical Investigation Section of *The Western Journal of Medicine*. Although the initial months were predictably slow, the flow of manuscripts in and out of my office the past six months has crescendoed nicely. In the first year we received and completely processed 30 manuscripts of which 50% were accepted. We published 14 articles (July and November 1983, February and March 1984). Most submissions were by researchers in California and Washington, the remainder coming from six other states. The manuscripts most frequently focused on areas in the subspecialties of infectious disease, endocrinology, cardiology and pulmonary disease. Thanks to a very energetic group of reviewers, the turnaround time for processing was quite short. On average, 31 days elapsed between the time the manuscripts were received in my office and the day I sent out the initial decision to the authors.

I conclude from all this that we are off to a fine start both quantitatively and qualitatively. If the crescendo persists, we will realize success in this venture by our second or third year. That will be sweet music, indeed. *The Western Journal of Medicine* will then be fully rounded out as a scientific medical journal and the Western Research Societies will feel justifiably proud of nurturing a valuable contribution to medical literature.

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Hepatitis B Virus Infection and Primary Liver Cancer—An Unfolding Story

THE REVIEW in this issue by Vyas and Blum on hepatitis B virus infection illustrates how rapidly modern methods in molecular biology can be utilized to increase our understanding of pathophysiologic principles underlying human disease. Before 1979 the recombinant cloning of DNA from viruses known to be pathogenic in humans (including hepatitis B virus [HBV]) was prohibited in the United States and most other countries where such experiments could be done. When recombinant DNA guidelines were revised to permit these experiments, HBV DNA was cloned immediately in four different laboratories and the complete DNA sequence was determined within six months.[1-6] The cloned DNA was then used as a hybridization probe to detect integrated HBV DNA in a hepatocellular carcinoma cell line derived from a hepatitis B surface antigen (HBsAg) carrier, PLC/PRF/5,[7-10] and within one additional year integration of HBV DNA was reported in cases of primary liver cancer from many patients all over the world who were HBsAg carriers.[10-13] Integration of HBV DNA was also found in nontumorous liver of patients who had hepatocellular carcinoma[14,15] and in the liver genome of carriers who did not have hepatocellular carcinoma.[13-18]

From many epidemiologic studies, cited by Vyas and Blum, it was well established that persistence of hepatitis B virus infection—that is, the carrier state—is associated with increased risk of hepatocellular carcinoma developing and now it was also evident from molecular studies that HBV DNA had the capacity to integrate into the host genome and that such integration occurred before development of hepatocellular carcinoma. This strengthened the link between hepatitis B virus infection and primary liver cancer. It represented the first clear demonstration of a pathogenic DNA virus integrating into the human genome in a native host and established that HBV had properties consistent with those which might be expected for an oncogenic virus.

Like animal DNA tumor viruses—such as SV 40 and adenoviruses—in which integration of viral DNA correlates with the transformed state of cells in culture,[19] integrated HBV DNA shows rearrangement of both viral and adjacent cellular sequences at the site of integration.[20-22] A most exciting recent finding has been the observation by Rogler and Summers[23] of possible intermediates in the integration process. Using woodchucks, which are naturally infected with a virus, woodchuck hepatitis virus (which is structurally, molecularly and pathophysiologically related to HBV), these investigators cloned and characterized various molecular forms of woodchuck hepatitis virus from carrier woodchucks. Hepatocellular carcinoma develops in these animals rapidly and with extraordinarily high frequency during persistent woodchuck hepatitis virus infection.[24] In hepatitis virus carrier woodchucks in which liver cancer has not yet developed, Rogler and Summers have identified high-molecular-weight nonintegrated viral molecules with extensive sequence rearrangement,[23] as well as integrated viral molecules with simpler viral genome patterns and no rearrangement of flanking cellular sequences.[25] The correlation of these molecules with sequential events in the integration process and cellular transformation awaits further study. Cloning and characterization of integrated HBV DNA molecules from human primary liver cancer and a variety of cell lines derived from such tumors is also now in progress.[20-22]

Another potential use of molecular hybridization technology is in the clinical laboratory both as a diagnostic tool and in understanding the pathophysiology of chronic liver disease associated with persistent

hepatitis B virus infection. In carriers who are HBsAg-positive and hepatitis B e antigen (HBeAg)-positive, the situation is relatively clear. These patients have continued replication of virus in their liver, associated with chronic liver disease (chronic persistent or chronic active hepatitis), and are potentially infectious to their intimate contacts. However, in carriers who are HBsAg-positive and anti-HBe-positive (HBeAg-negative), who have generally been considered to be past the stage of active virus replication, liver disease has been thought to be quiescent (either absent or at an end stage with development of cirrhosis) and there is little or no potential infectivity. Recent studies with cloned HBV-DNA probes, however, have shown that a significant number of HBsAg- and anti-HBe-positive patients retain viral DNA in the serum.[26-28] They also have continued virus replication in liver and active chronic liver disease and may indeed be infectious.[29] Some patients with antibodies to HBsAg in their blood (anti-HBs), who were formerly thought to have recovered from hepatitis B virus infection, also retain virus in their liver and this is sometimes in integrated form.[30] It has also been found that some patients with hepatocellular carcinoma who are anti-HBs-positive have integrated HBV DNA in their tumors.[14,15,30]

These rather alarming findings—which may represent the exception rather than the rule—indicate the limitations of standard viral marker testing (HBsAg and HBeAg). This is especially relevant when both viral antigens and antibodies or immune complexes are present in the blood. Some of these patients can be identified using radioimmunoassays with monoclonal antibodies to HBsAg, developed by Wands and co-workers.[31] However, another category of patients who have chronic hepatitis show negative results with all traditional serologic HBV tests, yet analysis of serum or liver DNA extracts reveals the presence of molecules hybridizing with HBV-DNA probes.[30,32] These patients would have formerly been considered to have non-A, non-B hepatitis. Some show positive serologic reactions with monoclonal anti-HBs[32,33] and are, therefore, considered to be infected with HBV or a related virus. Others may represent cases of persistent hepatitis B virus infection that is truly latent—that is, no viral gene products are produced—whereas a third category may represent entities that have not yet been fully defined.[33]

From histologic, cellular and molecular studies it appears that persistent viral hepatitis type B generally falls into two categories, permissive infection and nonpermissive infection.[29,34-36] A mixed type of infection may also be found in which features of replicating and nonreplicating infection are present in different cells or regions of the same liver. The features of replicating or permissive infection include active inflammatory liver disease (chronic persistent or chronic active hepatitis), HBsAg and hepatitis B core antigen (HBcAg) production by individual hepatocytes distributed randomly throughout the liver parenchyma and free virions and lower molecular weight replicating forms of HBV DNA. The features of nonpermissive infection include no active inflammatory liver disease (the liver may show no histologic evidence of hepatitis B virus infection other than the presence of "ground glass" hepatocytes or inactive cirrhosis resulting from viral infection many years earlier); continued HBsAg but not HBcAg production, and integrated HBV-DNA molecules. In some of the latter cases, HBsAg production is present in groups or clusters of hepatocytes that have the nodular appearance of a focal clonal growth. In several of the latter cases, we have noted integration of HBV DNA into unique host sites, again suggesting monoclonality of these cells.[29] In those patients who are no longer actively replicating virus (nonpermissive infection), regardless of whether or not HBsAg continues to be produced, the presence of integrated HBV DNA in a unique banding pattern may have important implications in terms of risk for future development of hepatocellular carcinoma.

The studies reviewed here suggest that integration of the HBV genome represents an important event which occurs during the transformation of hepatocytes. Regardless of whatever role HBV eventually is shown to have in the pathogenesis of hepatocellular carcinoma, molecular biological techniques will have in great measure contributed to that knowledge. The use of these powerful new methods will permit studies on the replication of the hepatitis B virus and possible elucidation of how this virus alters hepatocyte function during persistent infection. It is hoped that this will bring us to a better understanding of cellular and molecular events that lead to the development of hepatic malignancy.

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REFERENCES

1. Burrell CJ, Mackay P, Greenaway PJ, et al: Expression in *Escherichia coli* of hepatitis B virus sequences cloned in plasmid pBR-322. Nature 1979; 279:43-47

2. Charnay P, Pourcel C, Louise A, et al: Cloning in *Escherichia coli* and physical structure of hepatitis B virion DNA. Proc Natl Acad Sci USA 1979; 76:2222-2226

3. Sninsky JJ, Siddiqui A, Robinson W, et al: Cloning and endonuclease mapping of the hepatitis B viral genome. Nature 1979; 279: 346-348

4. Valenzuela P, Gray P, Quiroga M, et al: Nucleotide sequence of the gene coding for the major protein of hepatitis B virus surface antigen. Nature 1979; 280:815-819

5. Pasek M, Goto T, Gilbert W, et al: Hepatitis B virus genes and their expression in *E coli*. Nature 1979; 282:575-579

6. Galibert F, Mandart E, Fitoussi F, et al: Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in *E coli*. Nature 1979; 218:646-650

7. Marion PL, Salazar FH, Alexander JJ, et al: State of hepatitis B viral DNA in a human hepatoma cell line. J Virol 1980; 33:795-806

8. Chakraborty PR, Ruiz-Opazo N, Shouval D, et al: Identification of integrated hepatitis B virus DNA and expression of viral RNA in an HBsAg-producing human hepatocellular carcinoma cell line. Nature 1980; 286:531-533

9. Edman JC, Gray P, Valenzuela P, et al: Integration of hepatitis B virus sequences and their expression in a human hepatoma cell. Nature 1980; 286:535-538

10. Brechot C, Pourcel C, Louise A, et al: Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. Nature 1980; 286:533-535

11. Shafritz DA, Kew MC: Identification of integrated hepatitis B virus DNA sequences in human hepatocellular carcinomas. Hepatology 1981; 1:1-8

12. Gerin JL, Shih JW-K, Hoyer BH: Biology and characterization of hepatitis B virus, *In* Szmuness W, Alter HJ, Maynard JE (Eds): Viral Hepatitis, 1981 International Symposium. Philadelphia, Franklin Institute Press, 1982, pp 49-55

13. Koshy R, Maupas P, Miller R, et al: Detection of Hepatitis B virus-specific DNA in the genomes of liver cirrhosis tissues. J Gen Virol 1981; 57:95-102

14. Brechot C, Hadchouel M, Scotto J, et al: State of hepatitis B virus in hepatocytes of patients with HBsAg-positive and HBsAg-negative liver diseases. Proc Natl Acad Sci USA 1981; 78:3906-3910

15. Shafritz DA, Shouval D, Sherman HI, et al: Integration of hepatitis B virus into the genome of liver cells in chronic liver disease and hepatocellular carcinoma—Studies in percutaneous liver biopsies and post-mortem tissue specimens. N Engl J Med 1981; 305:1067-1073

16. Brechot C, Scotto J, Charnay P, et al: Detection of the hepatitis B virus DNA in liver and serum: A direct appraisal of the viral chronic carrier state. Lancet 1981; 2:765-768

17. Shafritz D: Hepatitis B virus DNA molecules in the liver of HBsAg carriers: Mechanistic considerations in the pathogenesis of hepatocellular carcinoma. Hepatology 1982; 2:35S-41S

18. Kam W, Rall LB, Smuckler EA, et al: Hepatitis B viral DNA in liver and serum of asymptomatic carriers. Proc Natl Acad Sci USA 1982; 79:7522-7526

19. Tooze J (Ed): Molecular Biology of Tumor Viruses, DNA Tumor Viruses, 2nd Ed, Part 2. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1980

20. Dejean A, Brechot C, Tiollais P, et al: Characterization of integrated hepatitis B viral DNA cloned from a human hepatoma and hepatoma derived cell line PLC/PRF/5. Proc Natl Acad Sci USA 1983; 80:2505-2509

21. Koshy R, Koch S, Freitag von Loringhoveir A, et al: Integration of hepatitis B virus: Evidence for integration in the single stranded gap. Cell 1983; 34:215-223

22. Rogler CE, Summers J, Shafritz DA: Molecular characteristics of hepatitis viruses in persistent infections of the liver and associated hepatic neoplasms, *In* Gallo R, Essex M (Eds): Cancer Cells, Vol 3. Cold Spring Harbor, NY, Cold Spring Harbor Laboratories, in press

23. Rogler CE, Summers J: Novel forms of woodchuck hepatitis virus DNA isolated from chronically infected woodchuck liver nuclei. J Virol 1982; 44:852-863

24. Snyder RL, Summers J: Woodchuck hepatitis virus and hepatocellular carcinoma. Cold Spring Harbor Conf Cell Prolif 1980; 7:447-457

25. Rogler CE, Summers J: Cloning and Structural Analysis of Integrated Woodchuck Hepatitis Virus Sequences from a Chronically Infected Liver. J Virol, in press

26. Bonino F, Hoyer B, Nelson J, et al: Hepatitis B virus DNA in the sera of HBsAg carriers: A marker of active hepatitis B virus replication in the liver. Hepatology 1981; 1:386-391

27. Weller IVD, Fowler MJF, Monjardino J, et al: The detection of HBV DNA in serum by molecular hybridization: A more sensitive method for detection of complete HBV particles. J Med Virol 1982; 9:273-280

28. Lieberman HM, LaBrecque DR, Kew MC, et al: Detection of hepatitis B virus DNA directly in human serum by a simplified molecular hybridization test: Comparison to HBeAg/anti-HBe status in HBsAg carriers. Hepatology 1983; 3:285-291

29. Hadziyannis SJ, Lieberman HM, Karvountzis GG, et al: Analysis of liver disease, nuclear HBcAg, viral replication and HBV DNA in liver and serum of HBeAg versus anti-HBe positive carriers of hepatitis B virus. Hepatology 1983; 3:656-662

30. Brechot C, Naples B, Courouce AM, et al: Evidence that hepatitis B virus has a role in liver cell carcinoma in alcoholic liver disease. N Engl J Med 1982; 306:1384-1387

31. Wands JR, Carlson RI, Schoemaker H, et al: Immunodiagnosis of hepatitis B with high-affinity IgM monoclonal antibodies. Proc Natl Acad Sci USA 1981; 78:1214-1218

32. Shafritz DA, Lieberman HM, Isselbacher KJ, et al: Monoclonal radioimmunoassays for hepatitis B surface antigen—Demonstration of HBV DNA or related sequences in serum and viral epitopes in immune complexes. Proc Natl Acad Sci USA 1982; 79:5675-5679

33. Wands JR, Lieberman HM, Muchmore E, et al: Detection and transmission in chimpanzees of hepatitis B virus-related agents formerly designated 'non-A, non-B' hepatitis. Proc Natl Acad Sci USA 1982; 79: 7552-7556

34. Shafritz DA, Hadziyannis SJ: HBV DNA in liver and serum, viral antigens and antibodies, virus replication and liver disease activity in patients with persistent Hepatitis B virus infection. *In* Chisari FV (Ed): Advances in Hepatitis Research. New York, Masson, 1984, pp 80-90

35. Hoofnagle JH: Chronic type B hepatitis (Editorial). Gastroenterology 1983; 84:422-424

36. Sherlock S, Thomas HC: Hepatitis B virus infection—The impact of molecular biology (Editorial). Hepatology 1983; 3:455-456

A New Ball Game

MEDICAL PRACTICE has become a new and more complicated ball game—one that seems to be played with at least four balls. To win, one must keep all four in the air at once. The four balls are (1) modern medical science and technology, (2) the physician as a caring person, (3) the business aspects of medical practice and (4) the responsibilities of a professional in a changing society. Each of these four is brought into play in the care of any patient in these parlous times. A practicing physician will need to keep an eye on them all and juggle them appropriately in the care of patients, no matter what the practice arrangements may be. There is reason to believe that we are apt to be best at medical science and technology, that there has been some slippage in our behavior as caring persons, that we have much to learn about the economic and business aspects of patient care and that we are only just beginning to be aware of our responsibilities as the most significant health care professionals in a changing society. Each of these four requires our close attention in this new game in which we find ourselves. And of course we should play to win.

There is nothing fixed or static about any of this. Everything is changing and always in motion. We must be sensitive, resilient, adept masters of our art and science in the interests of our patients and the public. This will not be easy to do. We are not alone in the field of health care. Nor are we necessarily loved by everyone. There are others ready to assume our role. But individually and as a profession we are still held in considerable esteem. We are still very much in this new ball game and so each of its four "balls" is briefly discussed below:

Modern Medical Science and Technology

This has been the main focus of our attention for some decades. Many of us have become more concerned with the diseases people have than with the people who have these diseases and who, being human, are afraid, in pain or wanting to feel better. Our success in applying modern medical science and technology in patient care has greatly increased life expectancy at both ends of the life span and improved the technical care of disease and injury at any age. This has been a source of great personal and professional satisfaction. But too many of us have thought too little about the financial cost of this to patients and to society. We have worshipped at the altar of modern medical science and technology. We have strived to keep up with changes in this and on the whole have done quite well. We do not practice as we were taught in our formal training. We use new knowledge and new technology as these become available. It is essential that we keep current with this progress and its uses in patient care. (Ball #1.)

The Caring Physician

Our dedication—and success—in the practice of good scientific medicine has too often been at the expense of attention to the very personal human interaction between doctor and patient. This interaction is expected and needed by a patient who is fearful, is in pain and does not understand everything about